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(54) Title: RAPID CELL PURIFICATION SYSTEMS

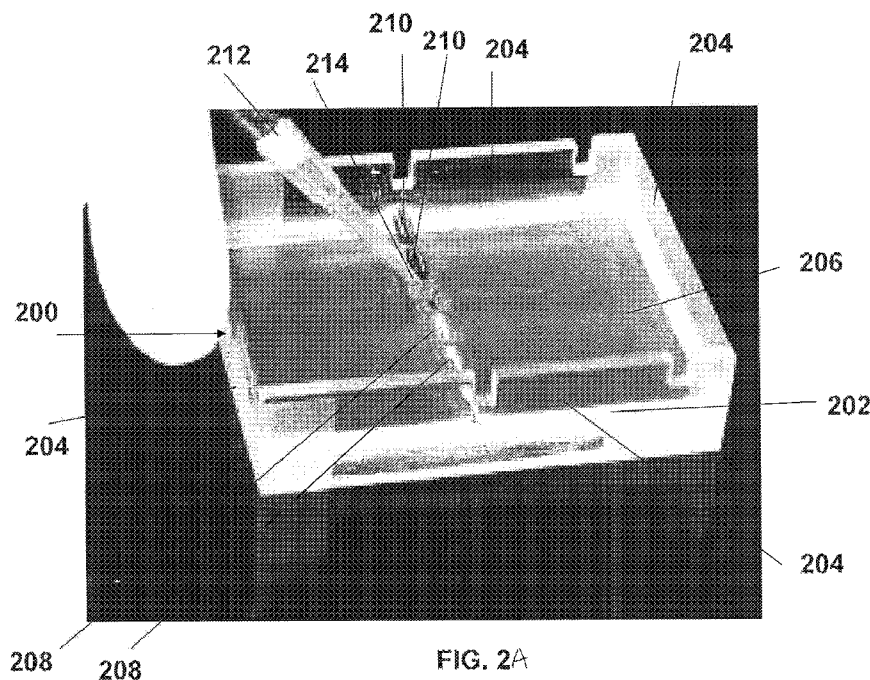


FIG. 2A

(57) Abstract: Methods and systems for purifying cells and viruses are provided. The sample is added to a well disposed in a medium. A potential is applied across the medium to cause the contaminants to enter one or more walls of the well, and retain the cells and/or viruses in the well. The cells and/or viruses can be removed from the well, and optionally adhered or fixed to a surface, or detected. In one embodiment, the cells and/or viruses may be retained in the well by embedding in the medium. The medium including the embedded cells and/or viruses may be excised or otherwise removed and transferred to a glass slide or other solid surface.

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- *as to the identity of the inventor (Rule 4.17(i))*
- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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RAPID CELL PURIFICATION SYSTEMS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This Patent Cooperation Treaty application claims priority to U.S. Application Number 61/449,824 entitled "Rapid Cell Purification Systems" and filed March 7, 2011, the contents of which are hereby incorporated by reference in their entirety.

FIELD

[0002] This disclosure relates to methods and systems for purifying cells and/or viruses, particularly microorganisms in a sample, particularly in preparation for diagnostics systems.

BACKGROUND

[0003] Diagnostic systems that detect cells and/or viruses is of clinical and diagnostic interest. Detection of cells and/or viruses is often prevented or complicated by the presence of contaminants that interfere with collection or detection of the cells and/or viruses. This may be particularly true for cells or viruses that are adhered or fixed to a solid surface prior to detection.

[0004] Additionally, operator variability may adversely impact the quality of specimen.

Specimen quality is dependent on patient factors including but not limited to differences between patients, and the presence or absence of various interfering substances. In many cases, the specimen is split and analyzed using various diagnostic tests. Therefore, purifying samples reliably and cost-effectively to remove in-homogeneities helps to improve the likelihood of relevant statistical sampling of cells and/or viruses therein.

SUMMARY

[0005] Methods and systems for purifying a microorganism are provided. The sample is added to a well disposed in a medium. A potential is applied across the medium to cause the contaminants to enter one or more walls of the well, and retain the cells and/or viruses in the well. The cells and/or viruses can be removed from the well, and optionally adhered or fixed to a surface, or detected. In one embodiment, the cells and/or viruses may be retained in the well by embedding in the medium. The medium including the embedded cells and/or viruses may be excised or otherwise removed and transferred to a glass slide or other solid surface. The medium may then be cut or sectioned to correspond to the respective wells. The medium is then dried, gram stained and the cells/viruses are detected.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] Fig. 1 is a perspective view of an embodiment of a system used to purify a microorganism.

5 **[0007]** Fig. 2A is a perspective view of an embodiment of a medium in a cassette which may be used with the system of Fig. 1.

[0008] Fig. 2B is a partial perspective view of another embodiment of a medium in a cassette which may be used with the system of Fig. 1.

[0009] Fig. 2C depicts a side view of the medium and system of Fig. 2B.

[0010] Figs. 2D and 2E illustrate excision of a portion of the medium of Fig. 2B.

10 **[0011]** Figs. 2F, 2G and 2H depict the excised portion of the medium of Figs. 2D and 2E on a solid surface.

[0012] Fig. 2I depicts gram stained microorganisms that may be detected in accordance with the systems and methods described herein.

15 **[0013]** Fig. 2J is a top view of a medium in a cassette which may be used with the system of Fig. 1, wherein a localization device is used to localize the cells and/or viruses.

[0014] Fig. 2K depicts gram stained microorganisms that may be detected in accordance with the systems and methods described herein.

[0015] Fig. 3 is a side view of an embodiment of a custom well-forming comb used to make wells in a medium.

20 **[0016]** Fig. 4 is a perspective view of multiple flow cell laminate assembly (top) and single flow cell cutaway with corresponding electrode and circuit details used to create a potential across a medium.

[0017] Fig. 5 is a graph showing results of accumulated objects (solid materials immobilized on a surface) over time for treated compared to non-treated samples.

25 **[0018]** Figs. 6A, 6B and 6C depict microscopic images of non-treated samples over time.

[0019] Figs. 7A, 7B and 7C depict microscopic images of treated samples over time.

[0020] Fig. 8 is a side view of an embodiment of a circular well formed in a medium with corresponding electrodes

[0021] Fig. 9 is a side view of an embodiment of a chamber formed in the medium with an inlet and outlet port enabling sample recirculation.

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DETAILED DESCRIPTION

[0022] Described herein are various embodiments of systems and methods for purifying cells and/or viruses in a sample. A sample containing cells and/or viruses is added to a well disposed in a medium. A potential is applied across the medium to cause contaminants to enter the medium through one or more walls of said well which retain the cells and/or viruses in the well. The cells and/or viruses are then removed from the well. The cells and/or viruses may also remain in or on the wall of the well, and/or the wall/well may be excised for further analysis.

[0023] The systems and methods described herein may concentrate cells and/or viruses from a low content specimen or sample in the wells thereby removing or reducing potentially interfering debris and resulting in more readable specimens. For example, the disclosed methods and systems may be used in testing of CSF (cerebro-spinal fluid) specimens or other hypocellular specimens. In such samples, bacterial organisms can be localized in 5x5 FOV capture areas (100x objective magnification) to minimize time-consuming searching during microscopic examination. A system having multiple wells may also be used to support parallel processing of sample aliquots for concurrent analyses by multiple downstream methods.

[0024] An exemplary embodiment of the system is depicted in Fig. 1. Fig. 1 depicts a system 100 used to purify cells and/or viruses with a cassette 106 configured to receive a medium (not shown). The cassette 106 includes a bottom plate 108 and sides 110. Negative electrode 102 and positive electrode 104 are operably connected to the medium through a buffer (not shown) placed in a reservoir 112.

[0025] The sample is added to a well disposed in a medium, preferably formed in the medium. In some embodiments, a plastic well may also be disposed in the medium, in addition to well(s) formed in the medium. An electrical potential is applied to the well causing contaminant material to enter the medium while the cells and/or viruses accumulate on the wall of the well. In some embodiments, the cells and/or viruses may be localized on the wall of the well. Cells and/or viruses remain in the well, thereby purifying the sample. The sample may be mixed during or after a time period of the applied electric field. In some embodiments, the process can be repeated until separation of contaminants that interfere with adhesion to a detection surface has been achieved. The well can then be rinsed, and cells and/or viruses

recovered. In some embodiments, the wall of the well where the cells and/or viruses have accumulated may be excised or otherwise removed from the rest of the gel medium. Alternatively, the electrical potential can be briefly reversed in polarity to displace the cells and/or viruses from the wall prior to rinsing and recovery. Mixing, applying a potential, and/or reversing polarity of applied field can be performed iteratively to further purify the sample.

[0026] In some embodiments, the sample volume recovered is less than, and sometimes substantially less than, the initial sample volume in the wells. In one embodiment, a barrier, such as an impermeable plastic sheet, is inserted into the wells and used to reduce the volume in the wells, thereby further concentrating the cells and/or viruses in the well and providing a reduced sample volume for recovery.

[0027] Systems, including electrophoresis boxes and electrodes can be obtained from Thermo Fisher, Waltham, MA, under the EC-Apparatus brand name having a product number of EC 250-90.

[0028] Fig. 2A depicts an embodiment of a medium in a cassette 200 comprising a bottom plate 202 and walls 204, which may be used with the system 100. The medium 206 is disposed in the cassette 200 showing a plurality of wells 208 in the medium 206. A sample 210 is added in some wells 208 with a pipette 212 comprising a pipette tip 214. Some empty wells 204 are also shown. Although the medium is shown as a top load gel slab, media in other forms, including but not limited to vertical gel slabs, can be used.

[0029] Fig. 2B illustrates an embodiment where the medium 206 is disposed in the cassette 200 showing a plurality of wells 208 in the medium 206. A sample is added and a potential is applied as described elsewhere herein to cause contaminant material to enter the medium while the cells and/or viruses accumulate or become embedded on the wall 209 of the well 208, as depicted in Fig. 2C. As shown in Figs. 2D-2E, a portion of the medium 206, including at least a portion of the wells 208, is excised, or otherwise removed. In some embodiments, the excision can be robotically automated.

[0030] Following excision, the excised portion of the medium may be fixed, for example, for staining or extracting molecular samples for analysis. In some embodiments, and as shown in Figs. 2F and 2G, the excised portion 211 may be placed on a solid surface 213, such as a glass slide and the excised portion 211 may be sectioned at each well. The sectioned, excised portion 211 may be dried, gram stained, and detected as indicated in Figs. 2H-2I. In some embodiments, a prepared slide (e.g. wherein the sectioned, excised portion has been dried) can be introduced into automated Gram staining equipment.

[0031] In some embodiments, the cells and/or viruses may be localized on the wall of the well by, or with the help of, a localization device 203. Figs. 2J and 2K depict one embodiment illustrating localization. Fig. 2J is a top view of a gel medium that utilizes non conductive materials as a localization device to distort the electric field resulting in localized concentration of cells and/or viruses, such as microorganisms. Fig. 2J illustrates an embodiment where the medium 206 is disposed in the cassette 200 showing a plurality of wells 208, including a plastic well 207, in the medium 206. The medium 206 includes a proximal end 206a and a distal end 206b. Disposed between the wells 208 and the medium 206 is a non-conductive material 203 including at least one hole or aperture 203a. A sample is added and a potential is applied as described elsewhere herein to cause contaminant material to enter the medium while the cells and/or viruses accumulate or become embedded on the wall 209 of the well 208. In this embodiment, the sample flows through the aperture 203a in the non-conductive material 203, thereby localizing the cells and/or viruses that accumulate or become embedded on the wall 209 of the well 208 as described with reference to Fig. 2C. In this embodiment, the non-conductive material prevents or inhibits the sample from flowing anywhere but through the aperture(s) 203a. In one embodiment, the non-conductive material may be a plastic film. As described above with reference to Figs. 2D-2I, a portion of the medium 206, including at least a portion of the wells 208, is excised, or otherwise removed. The excised portion may be placed on a solid surface 213, such as a glass slide and may be dried, gram stained, and detected. Fig. 2K depicts gram stained microorganisms 201 that have been localized in accordance with the methods and systems described herein.

[0032] While Figs. 2J and 2K depict one embodiment of a system and method for localization using a localization device 203 comprising non-conductive materials, other methods for localization and devices may also be used. In one embodiment, the localization device is a conductive material, such as a metal or metal alloy wire, that is embedded in or placed near a distal end 206b of the medium 206 (e.g. downstream relative to the direction of migration). When a potential is applied, the cells and/or viruses localize on the wall of the well in a location corresponding to the position of the conductive material. That is, where the conductive material is a straight metal wire, the cells and/or viruses localize on the wall of the well in a straight line corresponding to the line of the metal wire. In another embodiment, the localization device includes large and small (or discrete) electrodes are used. For example, a large electrode, such as a sheet electrode, may be placed at a proximal location relative to the proximal end 206a of the medium 206. A small electrode may be placed at a distal location relative to the distal end 206b of the medium 206. When a potential is applied, the cells and/or viruses localize on the wall of the well in a location corresponding to the location of the small electrode. In still other embodiments, the localization device is a discontinuous buffer system. In such a system, the conductivity inside the well is different from the conductivity outside the well. For example,

where a well is made of the medium, the conductivity of the sample and the conductivity of the well are different. When a potential is applied, the cells and/or viruses localize on the wall of the well due, at least in part, to this conductivity difference.

[0033] Dyes can be used in samples to pre-label or added to provide a tracking dye for purposes of a quantitative reference or sample transfer quality control indicator. Examples of dyes include colorants, bio-active adjuncts such as labeled antibodies, vital stains, mortal stains (such as propidium iodide and the like). Zwitterionic or neutrally charged dye molecules can be used to monitor electro-osmotic flow.

[0034] The potential applied across the medium can be applied for a variable time effective for removal of contaminants is dependent on the sample conductivity. For samples retrieved using normal saline and having a conductivity near that of normal saline, for example, the potential can be applied from 1 to 60 minutes.

[0035] In some embodiments, the method includes an asymmetric alternating potential. In other embodiments, the potential is a constant potential. In various embodiments, the applied potential induces electro-osmotic flow that is used to remove contaminants having a neutral charge. The potential can be reversed in polarity to displace cells and/or viruses from the surface of the medium. In some embodiments, the method includes applying a tangential flow to the medium to remove non-permeable contaminants from the surface of the medium. The tangential flow may be applied by flowing the sample over the medium. The tangential flow may be generated using additional buffer that is not the sample. The flow can be continuously cycled over medium.

[0036] When a sample is taken from a patient, there are various components in the sample. For example, in a patient suffering from pneumonia, a sample may include saline, anionic and cationic species, lung surfactants, bacteria, mucus, blood, host cells such as white blood cells, and/or lung tissue cells. Mucus components include but are not limited to mucoidal glycoproteins, proteins, extra-cellular nucleic acids, F-actin, lysed white blood cell fragments. Blood components may include but are not limited red cells, white cells, platelets, and plasma. Plasma components may include but are not limited to sugar, fat, protein and salt solution, platelets, blood clotting factors, sugars, lipids, vitamins, minerals, hormones, enzymes, antibodies, and other proteins including heme, albumins, immunoglobulins, fibrinogens, regulatory proteins, lipoproteins (chylomicrons, VLDL, LDL, HDL), transferrin, prothrombin, enzymes, proenzymes, residual antibiotics used to treat the patient, and hormones. Lung tissue components include host epithelial cells (intact or lysed). The cells in the alveolar walls of the lung, produce and secrete pulmonary surfactant. Pulmonary surfactant is a mixture of

phospholipids and proteins. White blood cells may also be present in lung samples. All the above components may be solubilized.

[0037] In some embodiments, the cells include blood cells, fungal cells, bacterial cells, or microorganisms including parasites. Examples of blood cells include red blood cells, and white blood cells. In some variations, the white blood cells can be neutrophils.

[0038] In various embodiments, microorganisms can include bacteria, fungi, algae, and protozoa. In one aspect, the microorganism is bacteria. The microorganisms can be pathogenic to humans and animals. Suitable microorganisms include any of those well established in the medical art and those novel pathogens and variants that emerge from time to time. Examples of currently known bacteria include, but are not limited to genera such as *Bacillus*, *Vibrio*, *Escherichia*, *Shigella*, *Salmonella*, *Mycobacterium*, *Clostridium*, *Corynebacterium*, *Streptococcus*, *Staphylococcus*, *Haemophilus*, *Neisseria*, *Yersinia*, *Pseudomonas*, *Chlamydia*, *Bordetella*, *Treponema*, *Stenotrophomonas*, *Acinetobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia*, *Citrobacter*, *Enterococcus*, *Legionella*, *Mycoplasma*, *Chlamydophila*, *Moraxella*, *Morganella*, and other human pathogens encountered in medical practice. Included in the genera are various species. For example, *Klebsiella* includes, but are not limited to *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Klebsiella oxytoca*, *Klebsiella planticola*, *Klebsiella terrigena*, and *Klebsiella ornithinolytica*. Examples of viruses include viroids.

[0039] Similarly, microorganisms may comprise fungi selected from a set of genera such as *Candida*, *Aspergillus*, and other human pathogens encountered in medical practice. Viruses can be, but are not limited to, orthomyxoviruses, (e.g. influenza virus), paramyxoviruses (e.g. respiratory syncytial virus, mumps virus, measles virus), adenoviruses, rhinoviruses, coronaviruses, reoviruses, togaviruses (e.g. rubella virus), parvoviruses, poxviruses (e.g. variola virus, vaccinia virus), enteroviruses (e.g. poliovirus, coxsackievirus), hepatitis viruses (including A, B and C), herpesviruses (e.g. Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus), rotaviruses, Norwalk viruses, hantavirus, arenavirus, rhabdovirus (e.g. rabies virus), retroviruses (including HIV, HTLV I and II), papovaviruses (e.g. papillomavirus), polyomaviruses, and picornaviruses, and the like.

[0040] The methods and systems described herein can be used to identify host cells harboring viruses. The cells are first purified, and subsequently the cells are manipulated to either produce viruses, or to identify nucleic acids in the cells.

[0041] The sample can be obtained from any number of sources, including, but not limited to, bodily fluids (including, but not limited to, blood, urine, serum, lymph, saliva, anal and vaginal

secretions, perspiration, peritoneal fluid, pleural fluid, effusions, ascites, and purulent secretions, lavage fluids, drained fluids, brush cytology specimens, biopsy tissue, explanted medical devices, infected catheters, pus, biofilms and semen) of virtually any organism, with mammalian samples, human samples, and environmental samples (including, but not limited to, air, agricultural, water and soil samples). In addition, samples can be taken from food processing, which can include both input samples (e.g. grains, milk or animal carcasses), samples in intermediate steps of processing, as well as finished food ready for the consumer. The method can be used for veterinary applications. The method can be also used for the analysis of milk in the diagnosis and treatment of mastitis, and the analysis of respiratory samples for the diagnosis of bovine respiratory disease. Furthermore, the method provides for the rapid detection of the presence of potential biological warfare agents in a sample.

[0042] Samples can range from less than a milliliter up to a liter for certain respiratory lavage fluids, and can further range in bacterial concentration from less than one bacterium to greater than 10^9 bacteria per milliliter. Furthermore, the sample can be present in blood, urine, sputum, lavage fluid or other medium. The sample can be concentrated before using the described methods for purifying cells and/or viruses from the sample. Sample concentration both concentrates the sample so that bacteria that are present in small numbers can all be effectively introduced into the system and adequately sampled, as well as so the background liquid medium can be normalized, or in some cases eliminated or reduced, to have consistent properties upon introduction to the system. Sample concentration can be performed by centrifugation, combining samples, removing solvents and the like. It should be noted, however, that certain samples provided in the description can be used without concentration or other modification .

[0043] The rapid detection of various cells and/or viruses is useful for a patient suffering from various diseases and disorders. For example, pneumonia can result from a variety of causes, including infection with bacteria, viruses, fungi, or parasites, and chemical or physical injury to the lungs. However, some samples of cells and/or viruses contain contaminants that interfere with the detection of such microbes. Purification of a microorganism (or virus or other cell) and detection of the type and amount of a microorganism (or virus or other cell) present is helpful to diagnose and treat a patient effectively.

[0044] In other embodiments, the cells are selectively lysed. For example in the case of intracellular targets, the mammalian cells can be lysed releasing intracellular microorganisms prior to, during, or after the purification described herein.

Contaminants

[0045] Contaminants are removed into the media. Contaminants that can be removed include ionic species, including but not limited to mono or divalent cations and anions, released intracellular materials, phospholipids, extracellular proteins, mucins, pulmonary surfactants, mucus plugs, pus, glycoproteins, and nucleic acids. In certain aspects of the purification methods, certain components such as cellular membrane fragments and larger cellular fragments are not removed from the media. Removing contaminants avoids other time intensive preparation steps such as vortexing and centrifugation. Cells and/or viruses remain in the well, and can be removed. In various aspects, the removed contaminants interfere with cells and/or virus surface immobilization, detection, and imaging.

Medium

[0046] Organogels, xerogel, and aerogels may also be utilized as the media. Aerogels include but are not limited to silica aerogel, carbon aerogels, alumina, cadmium selenide aerogels. Organic aerogels like SEAgel, made of agar. Aerogels made of chalcogens such as sulfur, selenium, and other elements may also be of utility.

[0047] In various embodiments, the medium is a hydrogel. In some embodiments, hydrogels are a network of polymer chains that are hydrophilic. Hydrogels can be highly absorbent natural or synthetic polymers, and in some instances can contain over 99% water. In general, hydrogels are solid, yet porous media.

[0048] The concentration of the hydrogel effects the migration speed of the contaminants through the hydrogel. Increasing the concentration of the hydrogel, decreases the pore size within the hydrogel. Additionally, contaminants with smaller molecules move faster and migrate further than contaminants with larger molecules.

[0049] The charge of the hydrogel also affects the migration speed of the contaminants through the hydrogel. Each contaminant molecule migrates to the electrode that carries a charge opposite to that of the contaminant molecule. Most biological materials have a net negative surface charge. Some have a net positive charge if the material has an excess of amines or other positively-charge moieties exposed to the surface. The charge is considered neutral if it is a balance of positive and negative, or uncharged, such as complexes coated with neutral materials that envelope and screen charged materials within. The uncharged material will migrate in the direction of electro-osmotic flow, if present.

[0050] The pH of the hydrogel also affects the migration speed of the contaminants and the targets. In some embodiments, the pH is selected to enhance mobility of the contaminants

relative to the cells and/or viruses. In some embodiments, a pH may be selected such that the cells and/or viruses are substantially near the isoelectric point minimizing the cells and/or viruses mobility relative to the contaminants. In other embodiments, the pH may be selected to be substantially different from the isoelectric point such that the direction of cells and/or viruses mobility is reversed relative to the contaminants.

[0051] In some embodiments, the media contains nutrients that promote the viability of the cells and/or viruses.

[0052] Media used in the systems described can separate contaminant molecules based on both their size and their charge.

[0053] The hydrogel's porosity is directly related to the concentration of agarose in the medium. Various levels of effective viscosity can be selected, depending on the experimental objectives.

[0054] Examples of hydrogels are alginates, as disclosed in Gadkari, 2007, "Optimal hydrogels for fast and safe delivery of bioactive compounds", Thesis of Drexel University; Ethyl-vinyl-acetate copolymer as disclosed in U.S. Pat. No. 3,854,480; esters of hydantoic acid as disclosed in U.S. Pat. No. 3,792,081, olefin saturated polyester 500-8000, polyethylene glycol (PEG) 200-1500, ethyl-vinyl-acetate copolymer 20-40% VA (20-30K), chlorinated polyethylene 25-45% Cl- (20-30K), ethyl-ethylacrylate copolymer 20-40% EA (20-30K), and ethylene vinyl chloride copolymer 25-45% Cl- (20-30K) as disclosed in U.S. Pat. No. 3,938,515; methyl-methacrylate copolymer, and glyceryl-methacrylate copolymer as disclosed in U.S. Pat. No. 3,957,362; ethylene-vinyl-acetate copolymer 4-80% VA (20-30K) as disclosed in U.S. Pat. No. 4,069,307; polysiloxanes as disclosed in U.S. Pat. No. 4,136,250; hydrophilic dihydroxyalkyl acrylate and insoluble copolymer as disclosed in U.S. Pat. No. 4,267,295; cellulose triacetate as disclosed in U.S. Pat. No. 4,220,152; acrylamide, vinylpyrrolidone, and polyethyleneoxide diol as disclosed in U.S. Pat. No. 4,423,099; poly-amino acid homopolymers and copolymers as disclosed in U.S. Pat. No. 4,351,337; poly-glutamic acid ethyl-glutamate copolymer (5-50% GA, 80-500KDa) as disclosed in U.S. Pat. No. 4,450,150; polyoxyethylene-polyoxypropylene copolymer thermoset as disclosed in U.S. Pat. No. 4,478,822; vinyl cross-linked copolymers of insoluble and soluble monoolefinic esters as disclosed in U.S. Pat. No. 4,548,990; copolymers with N-vinyl-2-pyrrolidone and methacrylates as disclosed in U.S. Pat. No. 4,693,884; polyanhydride as disclosed in U.S. Pat. No. 4,657,543; copolymer of poly(alkylene oxide) and cyclic ester of alpha hydroxy acid (glycolide) as disclosed in U.S. Pat. No. 4,882,168; polyacrylonitrile-nitric acid copolymer as disclosed in U.S. Pat. No. 5,218,039; N-morpholinoethyl methacrylate and 2-hydroxyethyl methacrylate copolymer as disclosed in U.S. Pat. No. 4,857,313; crosslinked copolymers of vinyl pyrrolidone and allylamine as disclosed in

U.S. Pat. No. 4,772,484; water soluble polyacetals having molecular weights from about 5,000-30,000 as disclosed in U.S. Pat. No. 4,713,441; thermoplastic hydrogels of polyvinyl pyrrolidone (PVP) and polyvinyl acetate (PVA), and gelatin as disclosed in U.S. Pat. No. 5,002,792; alginic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, Pectic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, hyaluronic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, polyglucuronic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, polymanuronic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, polygalacturonic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, polyarabinic acid with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, and kappa-carrageenan with Ca⁺⁺, Ba⁺⁺ or Zn⁺⁺, as disclosed in U.S. Pat. No. 5,089,606; charged side-chain polyphosphazenes with Ca⁺⁺ cross-linking as disclosed in U.S. Pat. No. 5,149,543; carboxymethylcellulose as disclosed in U.S. Pat. No. 5,208,037; agarose as disclosed in U.S. Pat. No. 3,961,628; and polyacrylamide as disclosed in U.S. Pat. No. 6,391,937; pluronic 127, N-isopropylacrylamide (NiPAM); and blends (block co-polymer, etc.), of all the above listed hydrogels.

[0055] Agarose is a linear polymer, made up of the repeating monomeric unit of agarobiose. Agarobiose is a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose. Agarose pectin or sulfonated agarose can be used as the hydrogel. Agarose can be obtained from Lonza, Rockland, ME under the brand name SeaKem TM. In certain embodiments, the concentration of the agarose gel for effectively removing contaminants is from 0.1-2.0% w/v.

[0056] Purified agarose hydrogels could be purchased for use in the described method. An example of a commercial purified hydrogel can be obtained from Invitrogen, Carlsbad, CA under the brand name E-Gel® EX Starter TM.

[0057] Polyacrylamide is a polymer (-CH₂CHCONH₂-) formed from acrylamide subunits. It can be synthesized as a simple linear-chain structure or cross-linked, typically using *N,N*-methylenebisacrylamide. In the cross-linked form, polyacrylamide is highly water-absorbent, forming a soft gel. Polyacrylamide can be obtained from BioRad, in Hercules, CA.

[0058] Purified polyacrylamide hydrogels could be purchased for use in the described method. An example of a commercial purified hydrogel can be obtained from BioRad, Hercules, CA.

[0059] Preconditioning of the media can be performed. Preconditioning of the media is often done to remove impurities found in the media. For example, providing a potential across a hydrogel helps mobile impurities to migrate outside of the hydrogel. The potential can be at least, and/or less than 50V, 75V, 100V, 150V, 200V, 250V, 300V, 350V, 400V or 500V. In various embodiments, the potential can be a period of time, such as at least 2 minutes, at least 5 minutes, at least 10 minutes, at least 15 minutes, at least 30 minutes, at least 45 minutes, at least 60 minutes, at least 120 minutes, or at least 180 minutes.

[0060] In various embodiments, the medium can be a filter. Examples of filters available from Pall Corporation, Port Washington, NY include hydrophilic polypropylene, which has characteristics of hydrophilic, low binding and pore sizes of 0.2 μm and 0.45 μm ; polytetrafluoroethylene (PTFE), which has characteristics of hydrophobic, high binding and pore sizes of 0.2 μm , 0.45 μm , 1 μm , 2 μm and 3 μm ; glass fiber, which has characteristics of hydrophilic, moderate binding and pore sizes of 1 μm ; nylon, which has characteristics of hydrophilic, low binding and pore sizes of 0.2 μm and 0.45 μm ; polyvinylidene fluoride PVDF, which has characteristics of hydrophilic, low binding and pore sizes of 0.2 μm and 0.45 μm ; PES (supor), which has characteristics of hydrophilic, low binding and pore sizes of 0.1 μm , 0.2 μm , 0.45 μm , and 0.8 μm ; vinyl/acrylic copolymer, which has characteristics of hydrophobic, for air sampling and pore sizes of 0.45 μm and 0.8 μm ; polyvinyl chloride (PVC), which has characteristics for air sampling and pore sizes of 5 μm ; hydrophilic mixed cellulose esters, which has characteristics of high binding and pore sizes of 0.45 μm ; hydrophilic acrylic copolymer, which has characteristics of pre-filter on support and pore sizes of 0.2 μm , 0.45 μm , 0.8 μm , 1.2 μm , 3 μm , and 5 μm ; nitrocellulose, which has characteristics of high binding and pore sizes of 0.2 μm . Examples of filters available from Millipore, Billerica, MA, include PTFE (LCR), which has characteristics of hydrophilic moderate binding and pore sizes of 0.2 μm and 0.45 μm ; PVDF (Durapore) hydrophilic, which has characteristics of low binding and pore sizes of 0.2 μm and 0.45 μm ; PTFE (Fluoropore), which has characteristics of hydrophilic, low binding and pore sizes of 0.2 and 0.45 μm ; nylon, which has characteristics of hydrophilic, low binding and pore sizes of 0.2 μm and 0.45 μm ; glass fiber, which has characteristics of hydrophilic, moderate binding and pore sizes of 1 μm ; hydrophilic mixed cellulose esters, which has characteristics of high binding and exemplary pore sizes of 0.2 μm , 0.45 μm , and 0.8 μm . Filters can have pore sizes of greater than 0.01 μm , 0.05 μm , or 0.1 μm , 0.2 μm , 0.4 μm , 0.6 μm , 0.8 μm , 1.0 μm , 1.5 μm , 2.0 μm , 2.5 μm , 3.0 μm , 4.0 μm , or 5.0 μm . Filters can have pore sizes of less than, or 5.0 μm , 4.0 μm , 3.0 μm , 2.5 μm , 2.0 μm , 1.5 μm , 1.0 μm , 0.8 μm , 0.6 μm , 0.4 μm , 0.2 μm , 0.05 μm , or 0.01 μm .

[0061] In various embodiments the method includes adding a chemical agent to the medium to increase the permeability of the medium and/or increase the ability of the contaminant to enter the medium.

[0062] Examples of chemical agents include reducing agents, including but not limited to dithiol threitol (DTT), tris(2-carboxyethyl)phosphine (TCEP), and mercaptoethanol reducing agents; denaturing agent using surfactants including but not limited to sodium laurel sulfates, non-ionic surfactants such as tritonx-100, tween-20, or chaotropic agents including but not limited to urea, thiourea, or guanidinium chloride; chelating agents that can coordinate molecules such as calcium, magnesium, and other divalent and trivalent ions also including

metal ions, using ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA); cleavage agents including proteases, nucleases, glyconases, lipases; and excipients such as poly ethylene glycol. In some embodiments a combination of one or more chemical agents can be utilized.

- 5 **[0063]** Viscous gels include cellulose ethers (such as hydroxyl ethyl cellulose or methocell) and soluble polymer viscosity modifiers (such as polyethylene glycol, polyvinylpyrrolidone, dextrans, pluronic surfactants, and alginates). In a viscous gel the pore size is not defined. The separation is based on retarded flow of the cells in the viscous media.

- 10 **[0064]** In some embodiments, agents can be added to or used to treat the medium to control electroosmotic flow. In some embodiments, it may be desirable to increase or decrease electroosmotic flow.

Sample Mixing

- 15 **[0065]** To analyze a representative sample, the sample is substantially uniform. In some embodiments the homogenization of a sample can be done by a sample mixing or stirring step. Mixing the sample acts to re-suspend any caked material formed on the walls of the well.

[0066] In various embodiments, the method includes mixing a sample using a pipette tip. See, for example, the pipette tip 214 in Fig. 2A. The sample is passed through the narrow pore opening of the pipette to shear and homogenize the sample.

Buffer Solutions

- 20 **[0067]** In various embodiments, the method includes placing a buffer in contact with the medium.

[0068] In some embodiments, the mixing parameters of the buffer are designed to maximize the removal of debris and non-target material.

- 25 **[0069]** In some embodiments, the buffer can be replenished to prevent accumulation of undesirable electrophoresis products. For example, undesirable effects pH gradients, generated at the cathode and anode and in proximity of the sample, can be substantially minimized by buffer replenishment or by replacement, potentially using continuous flow.

- 30 **[0070]** In various embodiments, numerous electrophoretic buffers utilize special pairs of redox mediators. In certain embodiments, these redox mediators allow low voltage electrophoresis to maintain cell viability, as well as the use of some electrode materials that have limited utility in high voltage electrophoresis (for example, indium tin oxide, "ITO" electrodes). In addition, these

redox mediators find use in "closed systems", e.g. systems not open to the atmosphere. In closed systems, bubble formation or other reactive species are not generated during the electrophoresis step, which can cause a number of problems, and secondly, closed systems also help to prevent the exposure of the technician to potentially infectious samples, as well as reduce problems associated with discarding biological samples

[0071] In some embodiments, the buffer is placed in a reservoir in contact with the medium. In various embodiments, the medium is not submerged in the buffer.

[0072] Buffers include, for example, various electrophoresis buffers including zwitterionic buffers, neutral buffers such as phosphate-buffered saline, (PBS), lower or higher pH buffers, hypotonic or hypertonic buffers. In some embodiments, borate and other selected ions and counter-ions are chosen for electrophoretic effectiveness.

[0073] In some embodiments, the buffer solution includes histidine and Tris (hydroxymethyl)-aminomethane. Histidine has low conductivity. Tris(hydroxymethyl)-aminomethane has some conductivity but has low mobility. Histidine has pKa values close to physiological values providing adequate buffering capacity. Tris(hydroxymethyl)-aminomethane can be obtained from Sigma-Aldrich, St. Louis, MO under the brand name Trizma® base (Sigma, T1503).

[0074] In some embodiments, the sample (in 150mM NaCl) is desalted removing cationic and anionic species that may interfere with subsequent analysis. In some embodiments, desalting allows successful concentration and capture of the microorganism.

[0075] Electrophoretic mobility can be buffer dependent due to zeta potential variability with salt concentration, valency of salts present in the buffer, pH of the buffer. Bacteria can lose charge as the concentration of salt increases or as the pH is lowered below a certain pH, for example pH 5.0. Divalent and trivalent salts are more effective quenchers than monovalent salts. For example, CaCl_2 is more effective than NaCl to quench a charge. Certain agents such as chelators, including but not limited too, Ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), both available from Sigma-Aldrich, St. Louis, MO, can be used to control the concentration of charged species in the sample.

Wells

[0076] As shown in Fig. 2A, one or a plurality of wells can be formed in the medium. The wells are molded into the gel. For example, a custom well-forming comb can be used to create the appropriate well shape. Wells include side-walls that can be substantially vertical or diagonal. In various embodiments the method includes wells that are non-rectangular shaped. In various embodiments the wells are substantially chamfered to eliminate sharp edges in the

well, enhancing target recovery. In some embodiments, the wells can hold various sample sizes. In various embodiments, the wells can hold from 10 μ L to 500 μ L. In some embodiments the wells are 5 to 250 mm wide. Multiple wells can be used for a sample. As shown in Fig. 9, the well may be circular surrounding an electrode and the counter electrodes may surround the well.

[0077] Fig. 3 depicts a side view of an embodiment of a custom well-forming comb 300. A comb body 302 has a plurality of well-forming teeth 304 connected to the comb body 302 by one edge 306. The sides of the teeth 308 form the side-walls of the non-rectangular shaped wells in a medium. In this embodiment the wells are triangular shaped. The comb 300 is sized and shaped to fit the medium in the cassette, see for example Fig. 2A. In this embodiment, the comb 300 has six well-forming teeth 304, but could have more or less teeth depending on the size of the cassette.

[0078] In a rectangular or square bottomed well, sample solution can wick up the walls of the well. In a triangular shaped well, the sample solution does not tend as strongly to wick up the walls, making it easier to remove the microorganism from the well. In some embodiments, the triangular shaped well is narrowest at the bottom and widest at the top of the well. The well-forming teeth 304 shown in Fig. 3, create a pattern of triangular shaped wells in the medium. In other embodiments, the wells may be round bottomed wells.

[0079] The samples can have high solids(e.g. from 1% - 50% weight / volume of solid components)/ Minimizing the well width minimizes the caking of the solids on the well walls. In some embodiments, the well is 0.0025 inches wide at the widest point.

Chambers

[0080] In various embodiments, the method uses a chamber wherein one or a plurality of chambers can be formed in the medium. The chambers are molded into the gel and have an inlet and outlet port as shown in Fig. 8. The chambers may be submerged or partially submerged in buffer.

Electrodes

[0081] In various embodiments of the method, an electrode or a plurality of electrodes may be contained within the well or chamber. Additionally, in various embodiments the electrode or plurality of electrodes may be in contact with the media or separated a distance from the media. The electrode or plurality of electrodes may be connected to the media using salt bridges, buffer, redox mediators, or other conductive charge transfer methods used by those skilled in

the art familiar with techniques used in applications establishing faradiac current. In some embodiments the electrodes are in physical contact with the chamber walls.

[0082] In various embodiments conductive materials may be utilized to distort the electric field resulting in localized concentration of cells and/or viruses. Electric field distortion may utilize material conductivity differences to accomplish the said localization.

Immobilization of the Cells and/or Viruses

[0083] In various embodiments the method includes immobilizing the microorganism. Cells and/or viruses are immobilized by various filters that exclude the targets (microorganism) from penetrating, for example, tube walls, microchannels (horizontal or vertical), or any geometry that uses a capture surface (specific or nonspecific), mazes, fluidic dead space (eddy cul-de-sacs), and microwells of approximate cellular scale.

Detection Surface

[0084] In some embodiments, the method includes that the cells and/or viruses are immobilized on a positively charged surface. For example, cells and/or viruses can be immobilized by a positively charged detection surface. In other embodiments, the cells and/or viruses may be immobilized by embedding in the medium.

[0085] Detection surfaces are disclosed in, for example, U.S. Patent No. 6,844,028, incorporated by reference herein in its entirety. Detection surfaces can include those coated with Poly-L-lysine, Poly ethyleneimine, or other cationic polymers. Additionally, detection surfaces can include hydrophobic surface coatings.

[0086] After the contaminants are removed from the sample by the medium, the microorganism can be detected by a system. In some embodiments, the system is an optical sensing system. In some embodiments, the system is a microscope.

[0087] In some embodiments, the system is an automated system.

[0088] In various embodiments, the sequential or simultaneous use of a plurality of electrophoresis electrodes allows multidimensional electrophoresis, i.e. the solution may be targeted, "mixed" or "stirred" in the vicinity of a detection surface, to further increase the kinetics of binding. For example, polarities can be reversed to allow cells and/or viruses that may not have bound to the detection surface to travel back "over" the surface, resulting in increased binding. Also, electrodes may be located and field polarity switched according to a programmed sequence so as to provide agitation in two dimensions of a plane, or in three dimensions.

Detection of the Microorganism

[0089] In various embodiments, the method includes detection of the microorganism. In general, biosensor devices are designed to fit into a detection unit, and generally utilize a number of components, which can either be "on-chip" (e.g. part of a biosensor cartridge) or "off-chip" (where some of the components are part of separate device or devices into which the biosensor cartridge fits). These components include, but are not limited to, one or a plurality (e.g. an array) of detection surface(s), concentration modules (which as outlined herein frequently is configured with the detection surface(s)), detection modules (again, frequently configured with the detection surface(s)), input and output ports, channels, pumps, mixers, valves, heaters, fluid reservoirs (including sample reservoirs, reagent reservoirs, and buffer reservoirs), concentration controllers (e.g. in the case of electrophoresis, electrical controllers), and data collection and analysis (e.g. computer) components.

[0090] An example of a microorganism diagnostic system is described in U.S. Patent Application No. 10/888,828 filed July 8, 2004, issued as U.S. Patent No. 7,687,239, and U.S. Application No. 11/303,803, filed December 16, 2005, issued as U.S. Patent No. 7,341,841, both of which are incorporated herein by reference in their entirety.

[0091] Low levels of cells and/or viruses can be detected with this method. Cells can be measure in terms of cells per mL, colony forming units (CFU, or units) per mL for fungi and/or bacterial microorganism, and or viruses can be measured in particles per mL or plaque forming units per mL (PFU). Levels of cells and/or viruses are described in units per volume, typically per mL volume. Those skilled in the art understand the specific units are typically reported and appropriate for a given target. For exemplary purposes, the concentration ranges below are reported in generic units per mL. For example, levels of 0.1 to 1×10^8 units/mL can be detected. In various embodiments, cells and viruses of levels less than 5×10^8 units/mL, 3×10^8 units/mL, 1×10^8 units/mL, 0.8×10^8 units/mL, 0.6×10^8 units/mL, 0.4×10^8 units/mL, 0.2×10^8 units/mL, or 0.1×10^8 units/mL can be detected.

EXAMPLES

[0092] The following examples are provided for illustration purposes and are not intended to limit its scope. Other variants will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

[0093] The example described below anticipates a wide range of specimen variability first homogenizing the specimen, then sampling the specimen, and then purifying the sample to remove debris and other interfering materials by placing the sample in a medium containing a

well and applying a potential laterally across the medium to retain cells and pass contaminants into the media.

Example 1. Purification of Bacteria Cells from Respiratory Specimens

Gel Preparation

- 5 **[0094]** 10 grams of agarose powder (SeaKem, LE Agarose) was mixed with a 1 L of buffered solution containing 100 mM histidine (Sigma, H8000) and 2.5 mM Trizma® base (Sigma, T1503). The final concentration of agarose slurry was 1.0% (W/V). The solution was boiled to melt the agarose powder and the molten agarose was stored in liquid form at 40° C until use.

Gel Casting

- 10 **[0095]** Those familiar in the art of gel slab electrophoresis recognize that solid inserts or combs are routinely used to create a void volume in a gel slab that is later utilized for sample loading. Gel electrophoresis combs are generally nominally 1-2 mm thick, capable of holding nominally 100 uL of sample volume. In this example, a custom equilateral V-shaped well was used. The well had sides 1 cm long and a thickness of nominally 0.6 mm (0.025"). The comb
15 was inserted into a gel box container (E-C Apparatus, EC 250-90) and box filled with the molten agarose submerging a portion of the comb. The agarose was allowed to cool to room temperature forming an agarose gel. The comb was removed from the solidified agarose and the void volume of the comb formed a well in the material. The V-shaped well enabled facile recovery of the sample volume, described in further detail below, from the well.

20 Pre Treatment of the Agarose Gel Media

- [0096]** The gel box containing the agarose media slab, having triangular wells, was placed in an electrophoresis apparatus and then submerged in a run buffer containing 100 mM histidine and 2.5 mM trizma base. A 250 volt potential was applied for 1 hr. The applied potential yielded 22 mAmps of current. The pre-treated gels were removed from the gel box and apparatus then
25 transferred to a closed container and stored submerged in fresh run buffer until use.

Specimen Homogenization

- [0097]** A remnant specimen having a known level of bacteria was homogenized by pouring into a syringe connected to 0.02" (0.5 mm) inner diameter peek tubing and forced through the peek tubing 10 times to liquefy the specimens at a flow rate of approximately 0.1mL/sec. The
30 specimen was then filtered through 5 um track etch polycarbonate filters (SPI Pore, E5013-MB).

A 1 mL sample aliquot of the specimen was processed as described below. An aliquot of the specimen was also reserved as a control.

[0098] A control or a known clinical sample (e.g., with a known concentration of bacteria) can be compared to the unknown sample.

5 **Assessment of the Sample**

[0099] The sample was diluted to a final nominal bacterial concentration of 1.5×10^3 CFU/mL. 50 uL of the diluted sample was plated in triplicate on Mueller Hinton Agar (MHA) and placed in the incubator overnight. The number of colonies counted on the overnight incubated plates divided by the plated volume and multiplied by dilution factor yielded the actual number of input *Klebsiella oxytoca* bacteria in CFU/mL.

[00100] The sample was diluted 10 fold and then the optical density read was acquired at 625 nm to assess the amount of particulate debris in the sample.

Sample Loading

[00101] The pre treated gels were placed on the gel box and apparatus, padded dry, and excess liquid was removed from the triangular wells using 0.2 mm thick flat capillary plastic pipette tips (Fisher 07-200-519). The well was filled with 20 uL sample of the homogenized specimen.

Sample Treatment

[00102] Histidine/Tris run buffer was added to the apparatus so that liquid level was below the top of the gel slab. The sample was electrophoresed for 5 minutes at 250 volts and the samples were hydrodynamically sheared by pipetting the sample volume up and down 5x using a capillary pipette tip. The samples were electrophoresed again for 5 minutes at 250 volts and the samples then hydrodynamically sheared by pipetting the sample volume up and down 5x using a capillary pipette tip.

Post Treatment Assessment of Spiked Sample

[00103] The treated sample was diluted to a final concentration of 1.5×10^3 CFU/mL. 50 uL of the diluted sample was plated in triplicate on Mueller Hinton Agar (MHA) and place in the incubator overnight. The number of colonies counted on the overnight incubated plates divided by the plated volume and multiplied by dilution factor yielded the actual number of *Klebsiella oxytoca* bacteria recovered in CFU/mL.

[00104] The treated sample was diluted 10 fold and then the optical density read was acquired at 625 nm to assess the amount of particulate debris remaining in the sample.

Results

	Pre Treatment Optical Density (OD)	Post Treatment Optical Density (OD)	Fold Cleanup
MEDIA METHOD	0.2841	0.065	4.35

5 Electrode Configuration and Circuit Details

[00105] The 20 uL of recovered sample volume was diluted with 40 uL of 10 mM ascorbic acid and then introduced into a flow cell (described below) for electrokinetic concentration.

[00106] For comparison purposes, a 20 uL of a non treated sample was diluted with 40 uL of 10 mM ascorbic acid and then introduced into a flow cell (described below) for electrokinetic concentration.

Flow Cell Construction

[00107] Flow cells were assembled from using a three-layer die-cut laminate flow cell assembly (DLE, Oceanside, CA), sandwiched between indium tin oxide (ITO) coated glass slide flow cell floor (Delta Technology, Stillwater, MN) and an indium tin oxide coated 5 mil polyester (ITO PET) plastic flow cell ceiling (Sheldahl, Northfield, MN) forming a fluidic flow cell chamber. The laminate contained 32 separate channels, each having 1.78 mm width x 0.30 mm height x 11.28 mm length, with 1.78 mm diameter fluidic inlet and exit ports to interface with plastic pipette tips for fluid exchanges using manual pipettors. The transparent electrodes top and bottom electrodes enabled microscope imaging as illustrated in Fig. 4.

Fig. 4 is a perspective view of multiple flow cell laminate assembly (Top) and single flow cell cutaway with corresponding electrode and circuit details. Bacterial suspensions in redox active EKB were contacted with uniform transparent electrodes constructed from transparent indium tin-oxide (ITO) coated glass (Delta Technologies, Stillwater, MN) or polyester film (Sheldahl, Northfield, MN). A potential was applied to the conductive ITO surfaces completing the circuit,

establishing faradaic current, and an electric field between the electrodes enabling the bacterial electrokinetic concentration (EKC) and surface immobilization.

Bacterial Suspension and Surface Concentration Experiments

5 [00108] Studies were performed by loading the flow cells with a samples, with the power supply turned off, and then inserted onto the microscope stage. The microscope acquired images at the bottom flow cell surface during subsequent steps. The power supply was connected and cells electrokinetically concentrated to the flow cell's bottom surface by application of a 1.4V DC fixed potential. The top electrode (flow cell ceiling) was connected to the negative power supply terminal, and the bottom electrode (flow cell floor) was connected to 10 the positive terminal. The applied potential resulted in complete bacterial concentration in less than 3 minutes. After 300 seconds a -1.0 V DC fixed potential was applied for an additional 60 seconds to measure the degree of irreversible binding of the sample debris and bacteria on the flow cell floor. The digital microscope acquired images every 3-7 seconds during concentration.

Digital Microscopy Setup

15 [00109] An Olympus IX-71 inverted microscope equipped with a 12-bit, 1200 x 1600 pixel array monochrome CCD digital camera (MicroFire, Leeds Precision Instruments) was used for image acquisition. The transmitted illumination cone, created with an IX-PH3 annular ring placed in a 0.55 NA transmitted light condenser, was 33.4° from the normal to the microscope's focal plane. The illumination cone, after refraction through the flow cell's air-glass-ITO-liquid interfaces 20 (described below), resulted in a 24.5° forward scatter angle-of-incidence relative to the focal plane normal. The forward scatter angle-of-incidence relative for the flow cell's air-plastic-ITO-liquid interfaces was not calculated. In all formats, a dark image was obtained in the absence of scatterers, as the illumination cone passed outside the 20x, 0.4 NA microscope objective's (LCPlanFI Olympus, Leeds Precision Instruments) imaging cone. The presence of scatterers 25 resulted in the appearance of bright objects on a dark image background (dark-field image for objects within the focal depth). The system field-of-view was 444 x 592 µm with corresponding 0.37 µm pixel resolution. The imaging system's depth-of-focus and image depth were 5.8 µm and 3.8 mm respectively. Constant camera exposure and gain settings were maintained when relative intensity comparisons were performed as in the case of growth experiments described 30 below.

Accumulation Time Results

[00110] Fig. 5 is a graph showing results of accumulated objects over time for treated compared to non-treated samples. The non-treated sample data is expected data.

[00111] The objects are solid material, such as cells, viruses, and cellular debris, that are immobilized on a sample surface. Fig. 5 shows that material concentrates, then adheres to the surface. Subsequent processes such as measuring the growth or growth rate can be utilized to determine the number of viable cells, or additionally probing the material using receptor-ligand binding techniques including but not limited to antibody recognition or nucleic acid hybridization methods can be used to measure the abundance of microorganisms present.

[00112] Figs. 6A, 6B and 6C depict microscopic images of non treated samples at initial time, time of 300 seconds and time of 360 seconds, respectively. The surface accumulation rate is low. As shown in Figs. 6A, 6B and 6C, poor surface retention of the objects occurs when samples are not treated.

[00113] Figs. 7A, 7B and 7C depict microscopic images of treated samples at initial time, time of 300 seconds and time of 360 seconds, respectively. The treated sample surface accumulates all objects as evidenced by plateau occurring in less than 3 minutes. As shown in Figs. 7A, 7B and 7C, the objects were irreversibly bound to the surface as evidenced by consistent accumulated counts during reverse electrophoresis.

Bacterial Growth

[00114] After cell immobilization, the flow cell was rinsed with 10 times the internal cell volume with 1/10th strength cation-adjusted Mueller-Hinton Broth (CAMHB) growth media (Difco, Sparks, MD). 100 uL of liquified Mueller Hinton Agar (MHA) was loaded into the flow cell and the cooled to room temperature solidifying into a hydrogel.

Time Lapse Imaging

[00115] Direct observation of bacterial growth was performed by inserting the disposable 32-channel flow cell assembly into a custom bench-top automated instrument that combined digital microscopy, motion control, and image analysis software. The system was enclosed in an incubator maintained at 35°C. The motorized microscope stage enabled automated XY translation and location logging and memory with 10 μ m repeatability. The system automatically focused and acquired surface images of adherent bacteria at programmed time intervals for multiple fields-of-view during an experiment. The system used the fiducial markings to autofocus and mechanically align (± 1 pixel) the fields-of-view prior to image acquisition. Unless

stated otherwise, a single field-of-view contained sufficient numbers of cells for analysis and automated analysis routines counted the number of growing clones.

Growth Results

[00116] The number of growing clones observed using the digital microscope method was compared with the number of expected growing clones, assuming 100% yield and a 1 to 1 correlation between growing colonies on MHA plates, to calculate a digital microscopy method efficiency. The media method was compared to a alternative media method wherein the gel was submerged. A total efficiency was calculated by multiplying the treatment recovery and digital microscopy efficiency.

	Post Treatment Recovery	Digital Microscopy EFF	Total EFF
Control - No Prep	100%	12%	12%
Media Method	82%	90%	74%
Submerged Media Method	43%	100%	43%

The total efficiency for the media method was highest when the gel slab was not submerged.

[00117] All directional references (e.g., proximal, distal, upper, lower, upward, downward, left, right, lateral, front, back, top, bottom, above, below, vertical, horizontal, clockwise, and counterclockwise) are only used for identification purposes to aid the reader's understanding of the present invention, and do not create limitations, particularly as to the position, orientation, or use of the invention. Connection references (e.g., attached, coupled, connected, and joined) are to be construed broadly and may include intermediate members between a collection of elements and relative movement between elements unless otherwise indicated. As such, connection references do not necessarily infer that two elements are directly connected and in fixed relation to each other. The exemplary drawings are for purposes of illustration only and the dimensions, positions, order and relative sizes reflected in the drawings attached hereto may vary.

[00118] Numerous and varied other arrangements can be readily devised by those skilled in the art without departing from the spirit and scope of the description. Moreover, all statements

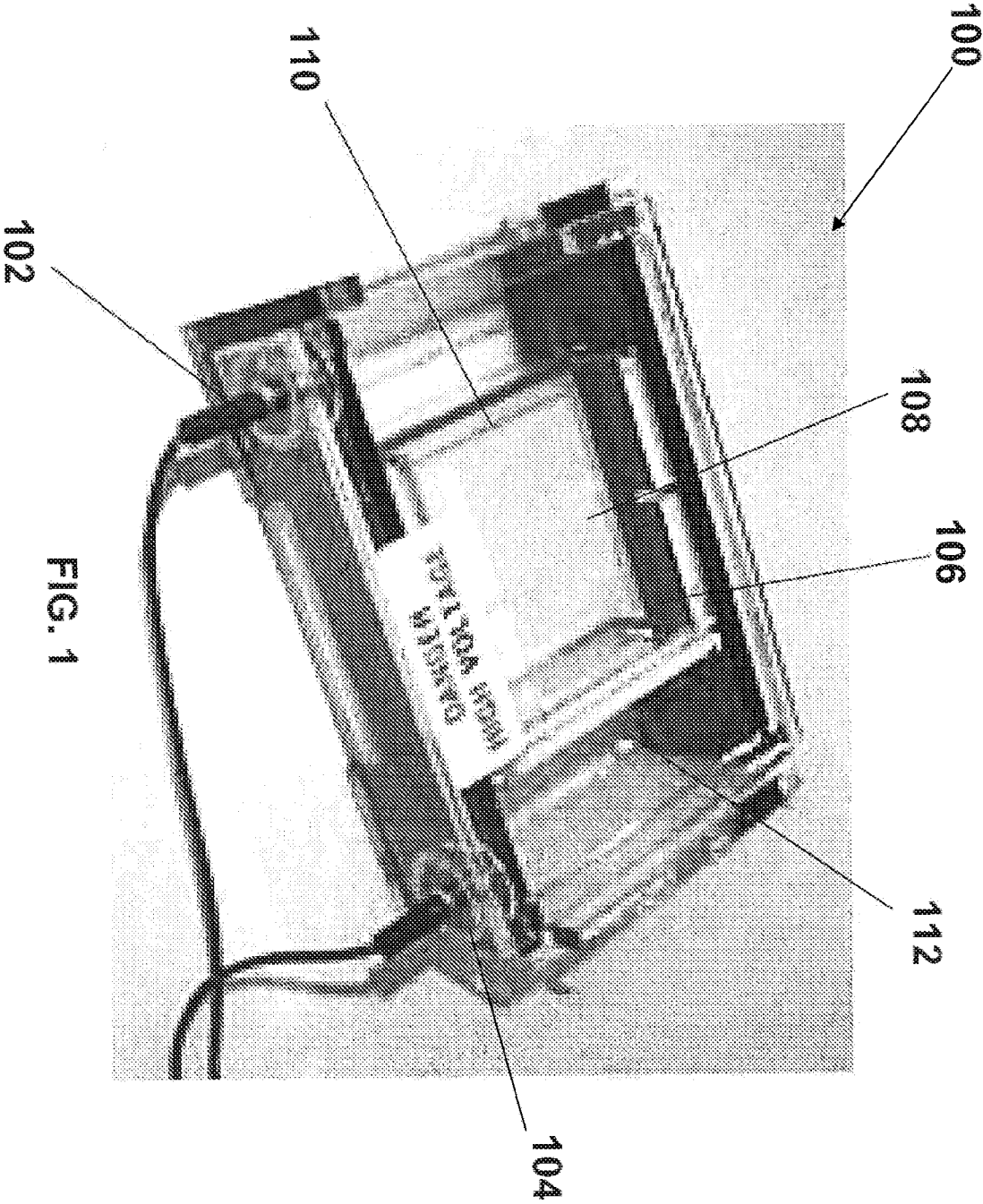
herein reciting principles, aspects and embodiments, as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents as well as equivalents developed in the future, i.e. any elements developed that perform the same function, regardless of structure. All references cited herein are incorporated by reference in their entirety.

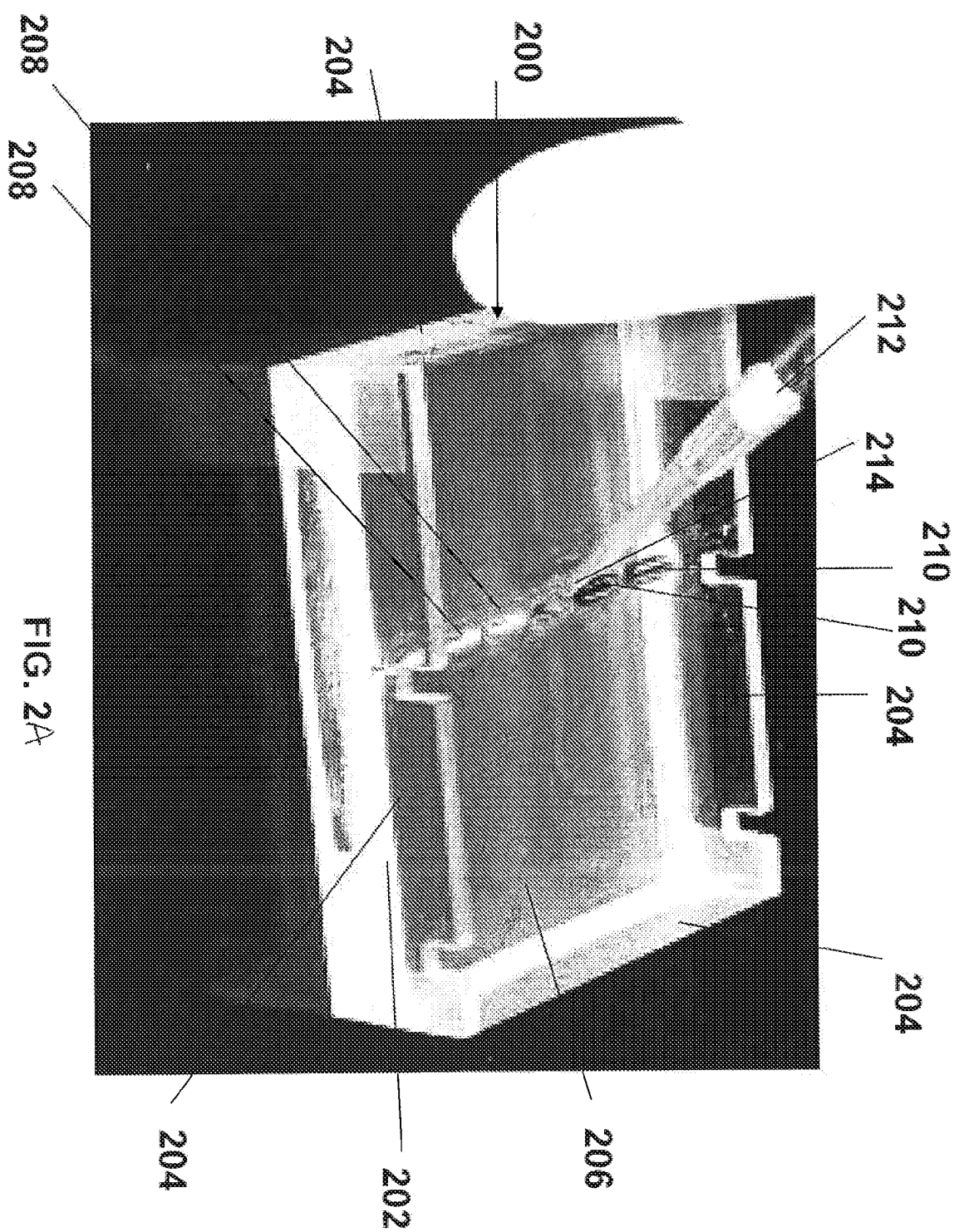
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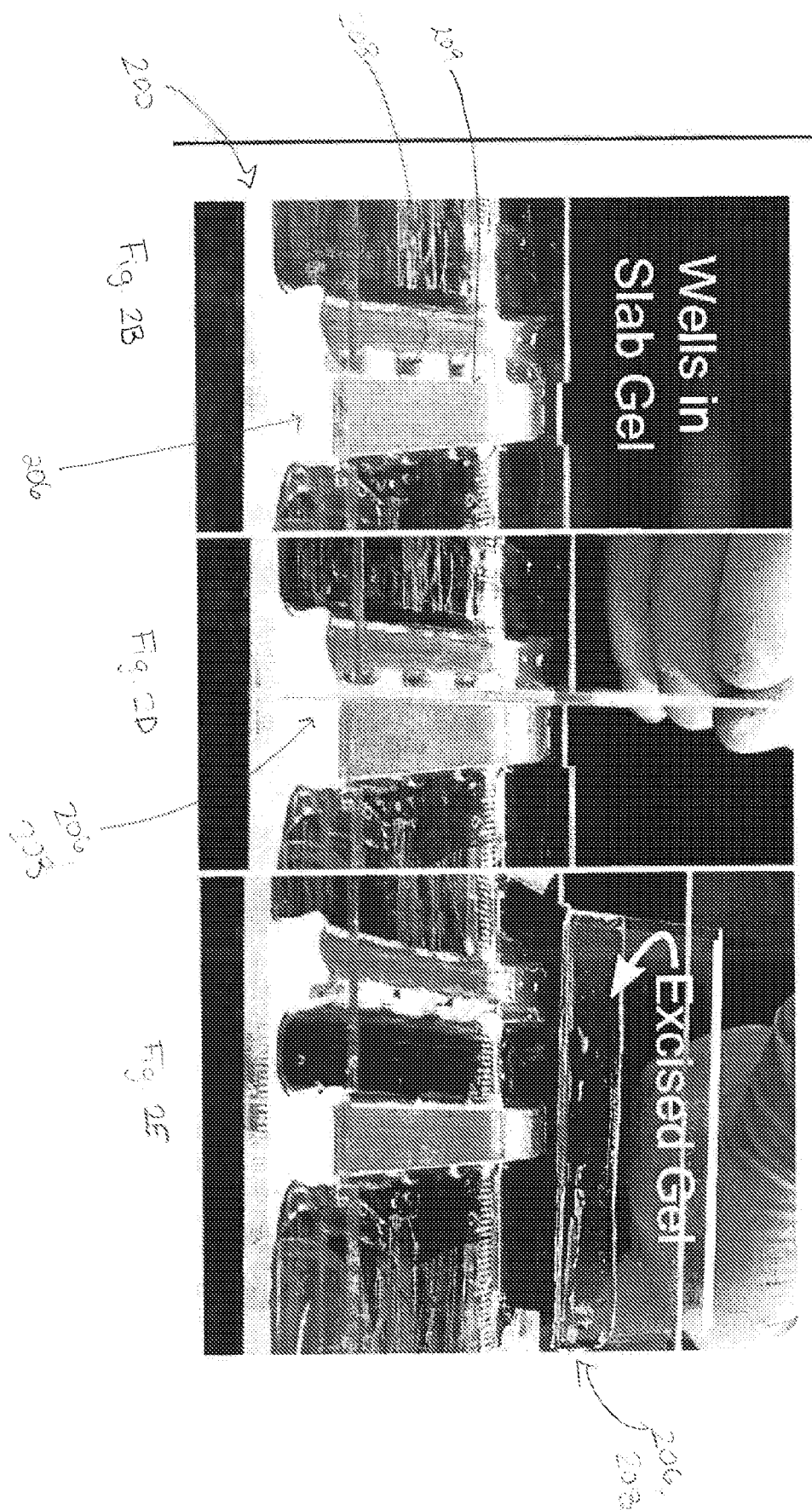
What is claimed is:

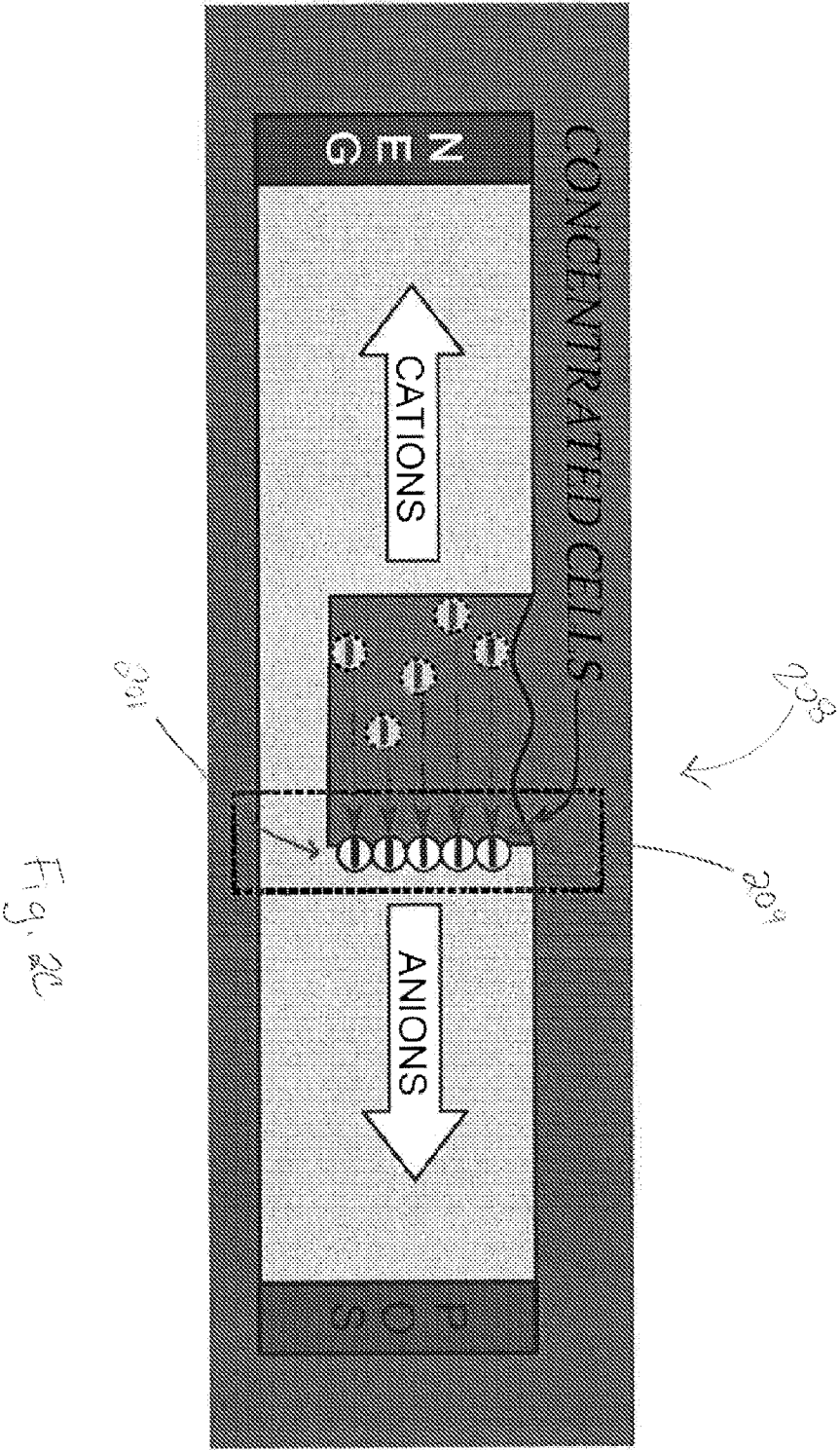
1. A method of purifying cells and/or viruses in a sample comprising:
adding the sample to a well disposed in a medium;
applying a potential across the medium to cause contaminants to enter said
medium through one or more walls of said well while retaining the cells and/or viruses in
5 said well; and
removing the cells and/or viruses from said well.
2. The method of claim 1, wherein said cells are microorganisms.
3. The method of claim 2, wherein said cells are blood cells.
4. The method of claim 3, wherein the blood cells are neutrophils.
5. The method of claim 2, wherein said microorganisms are bacteria.
6. The method of claim 1, wherein the cells and/or viruses are viruses.
7. The method of claim 1, wherein said cells are eukaryotic cells.
8. The method of any one of claims 1-7, wherein said medium is a filter.
9. The method of any one of claims 1-7, wherein said medium is a hydrogel.
10. The method of claim 9, wherein said hydrogel comprises polyacrylamide or agarose.
11. The method of any one of claims 1-10, comprising placing a buffer in contact with said
medium.
12. The method of claim 11, wherein said buffer comprises histidine and Tris
(hydroxymethyl)-aminomethane.
13. The method of any one of claims 1-12, further comprising immobilizing said cells and/or
viruses.
14. The method of claim 13, wherein said cells and/or viruses are immobilized on a positively
charged surface.
15. The method of any one of claims 1-14, further comprising detecting said cells and/or
viruses.
16. The method of any one of claims 1-15, further comprising adding a chemical agent to
the sample to increase the permeability of the contaminant into the medium.
17. The method of any one of claims 1-16, further comprising applying a tangential flow to
the medium to remove non-permeable contaminants from the medium.
18. The method of any one of claims 1-17, wherein the potential is one of an asymmetric
alternating potential or a constant potential.
19. The method of any one of claims 1-18, further comprising reversing the potential to
remove cells and/or viruses and contaminants from the surface of the medium.
20. The method of claim 1 wherein removing the cells and/or viruses from the well comprises
removing at least a portion of the medium including a wall of the well having the cells and/or
viruses accumulated thereon.

21. The method of claim 20 further comprising fixing the portion of the medium for staining or extracting molecular samples for analysis.
22. The method of claim 1 further comprising providing a localization device, wherein the localization device draws the cells and/or viruses to a discrete location in the well thereby localizing the cells and/or viruses.
23. The method of claim 22 wherein the localization device is a non-conductive material.
24. The method of claim 23 wherein the non-conductive material is plastic.
25. The method of claim 22 wherein the localization device is a conductive material.
26. The method of claim 25 wherein the conductive material is a metal wire or a metal alloy wire.
27. The method of claim 22 wherein the localization device comprises at least two electrodes.
28. The method of claim 27 wherein the at least two electrodes comprises at least one sheet electrode.
29. The method of claim 22 wherein the localization device is a discontinuous buffer system.









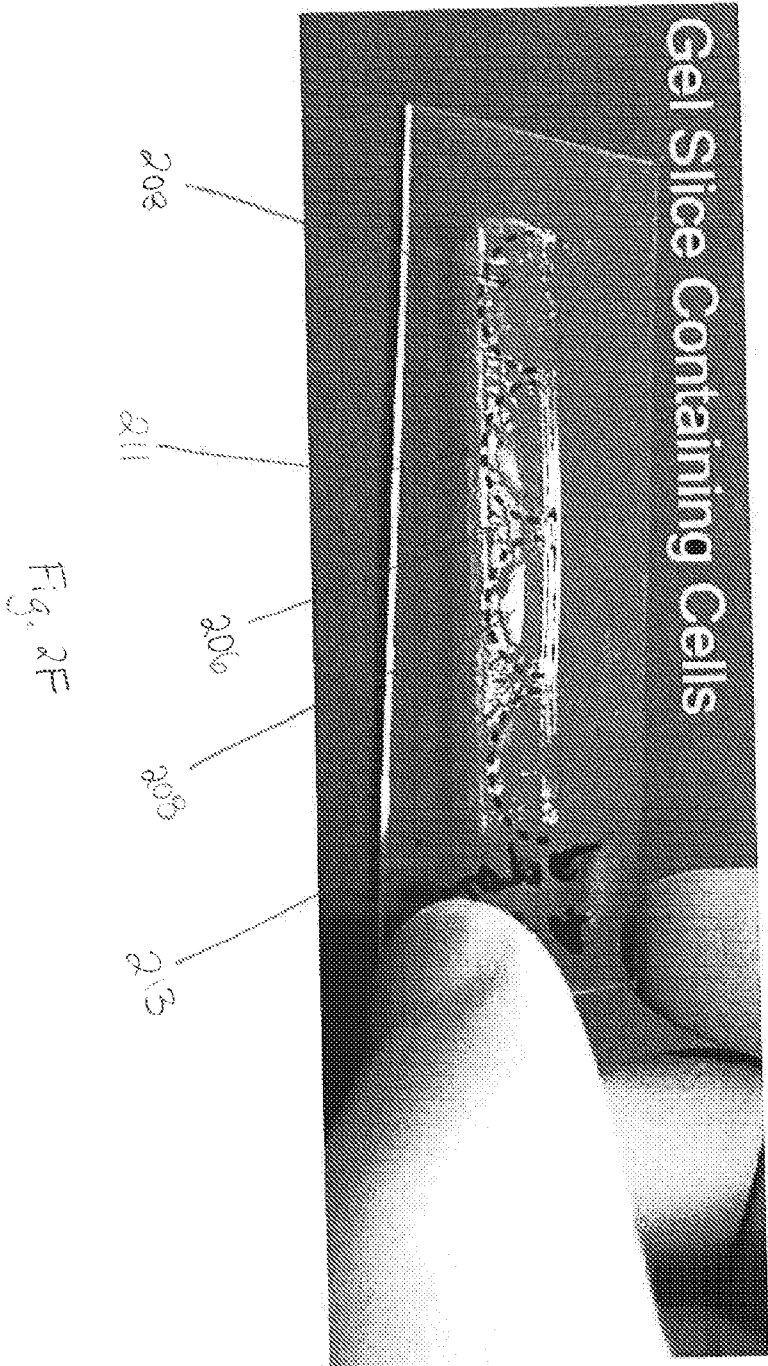


Fig. 26

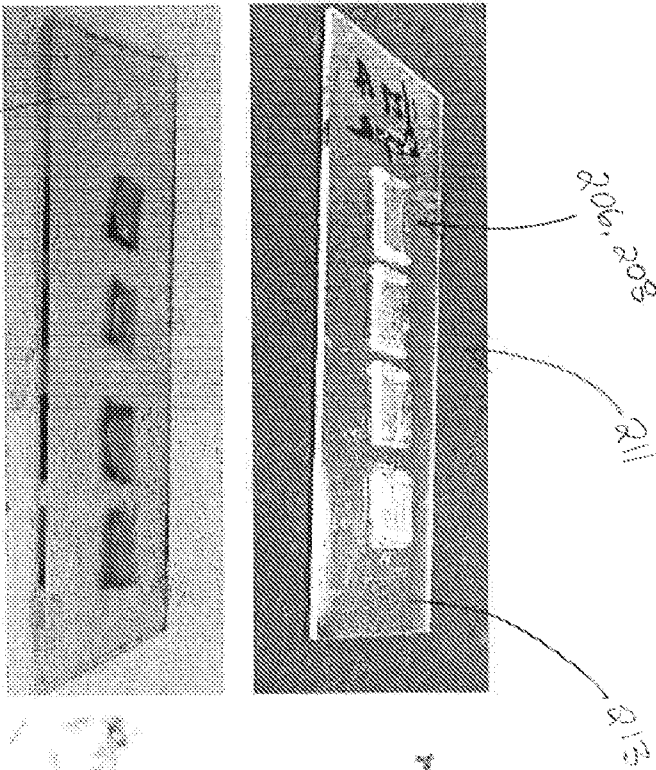


Fig. 24

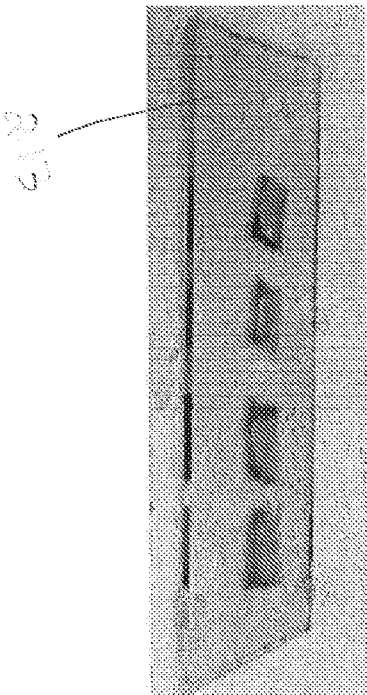


Fig. 25



FIG. 2J

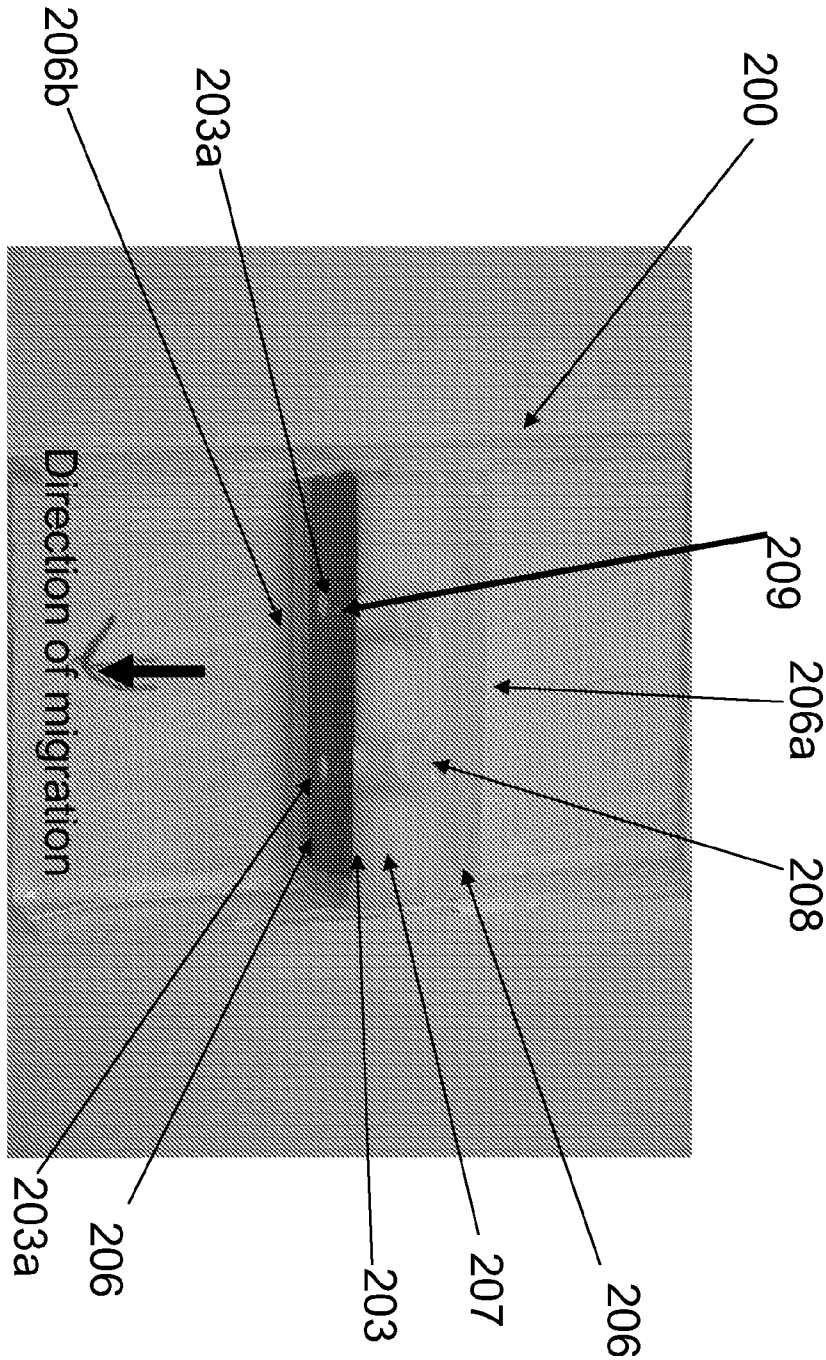
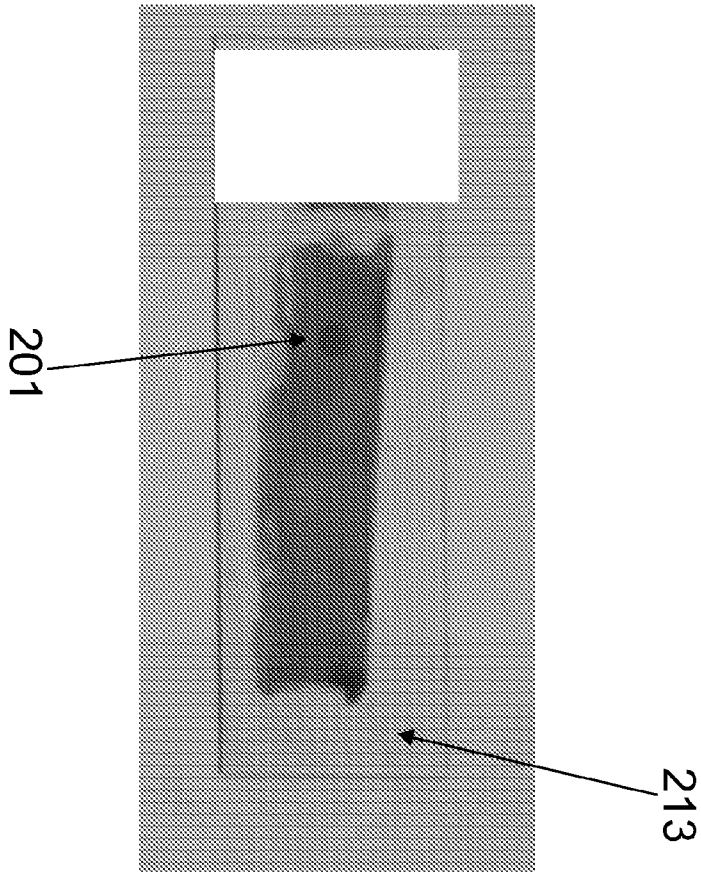


FIG. 2K



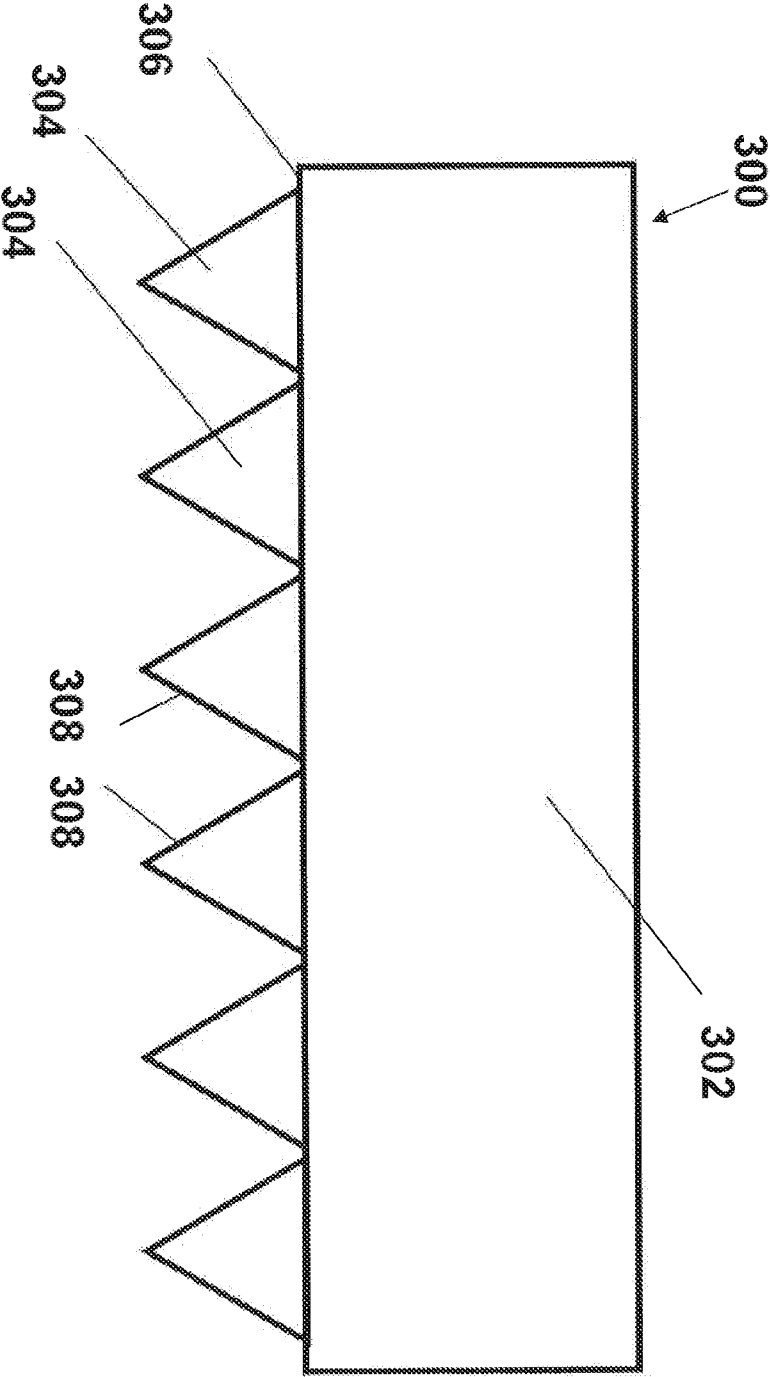
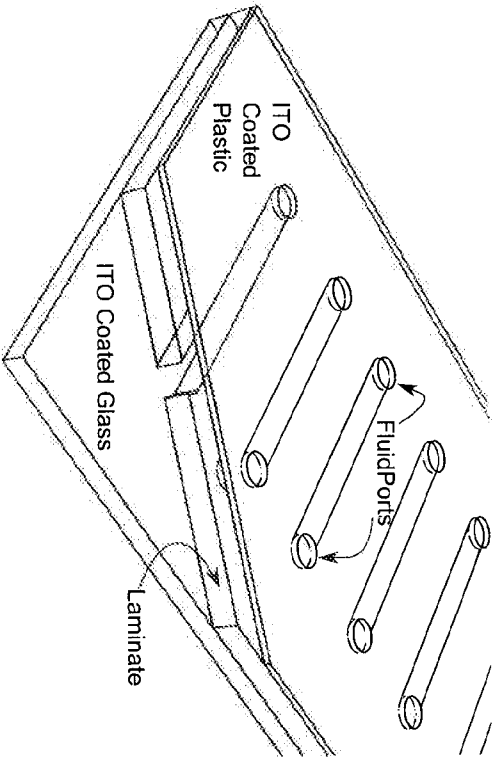


FIG. 3



Single Flow Cell Cutaway

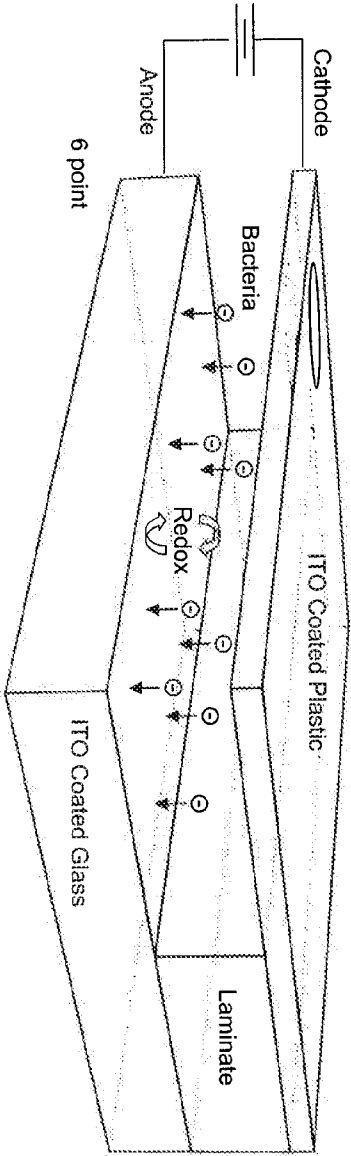


FIG. 4

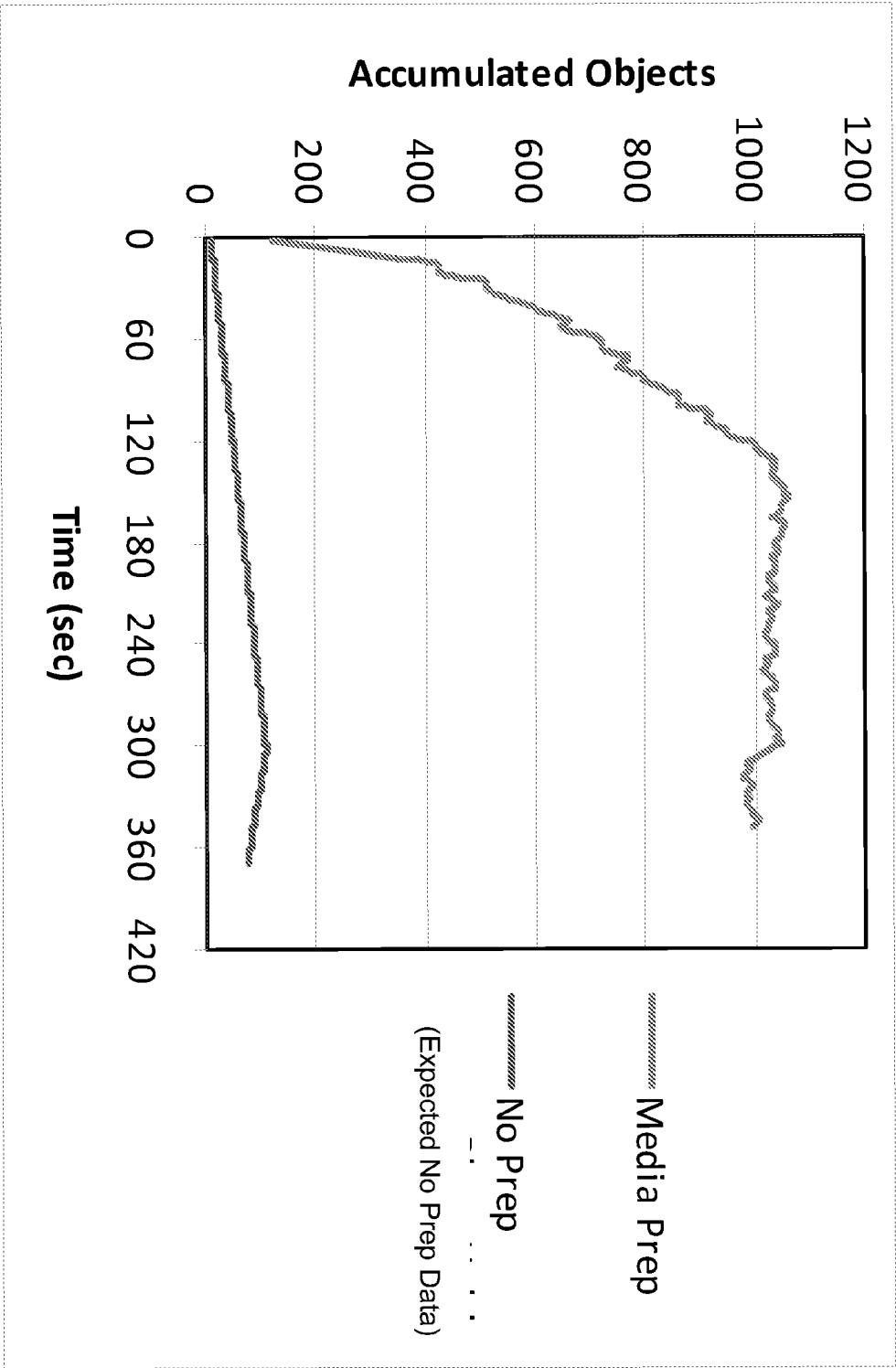


FIG. 5

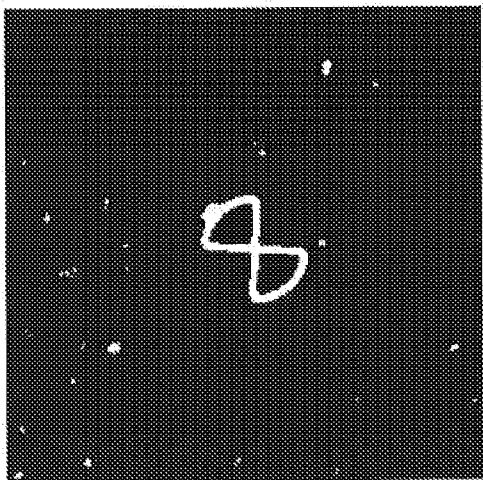


Fig. 6A

Cropped portion of microscopic image
of NON TREATED sample at $T = 0$

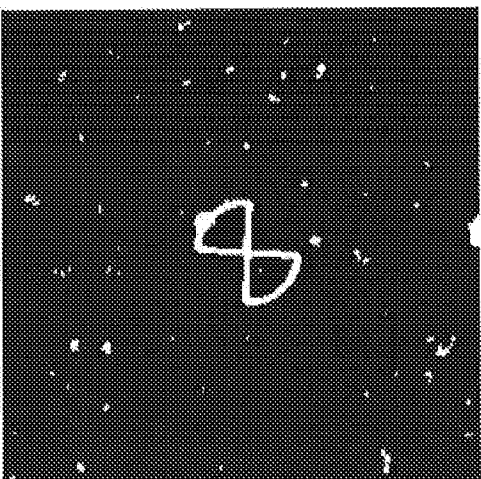


Fig. 6B

Cropped portion of microscopic image
of NON TREATED sample at $T = 300$

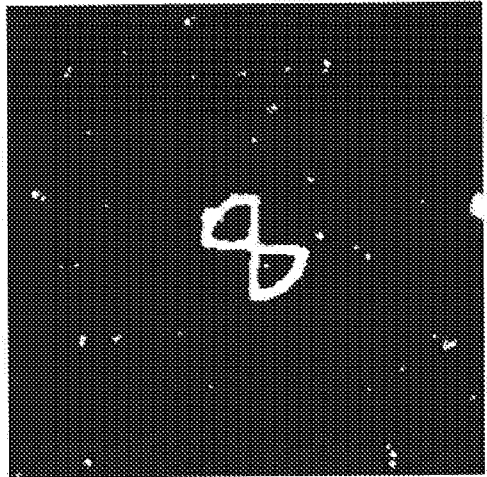


Fig. 6C

Cropped portion of microscopic image
of NON TREATED sample at $T = 360$
(poor retention of material)

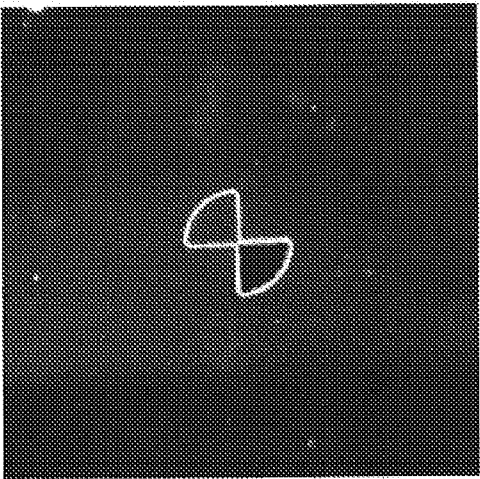


Fig. 7A

Cropped portion of microscopic image
of TREATED sample at $T = 0$

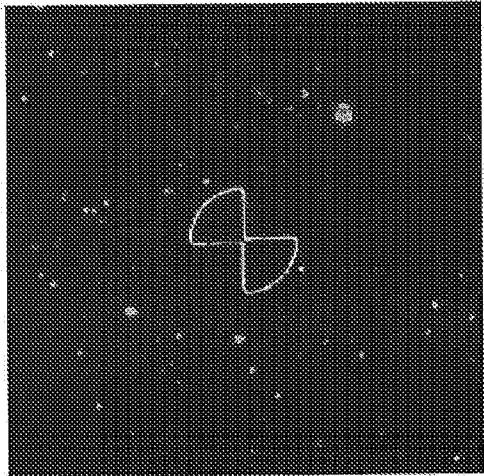


Fig. 7B

Cropped portion of microscopic image of
TREATED sample at $T = 300$

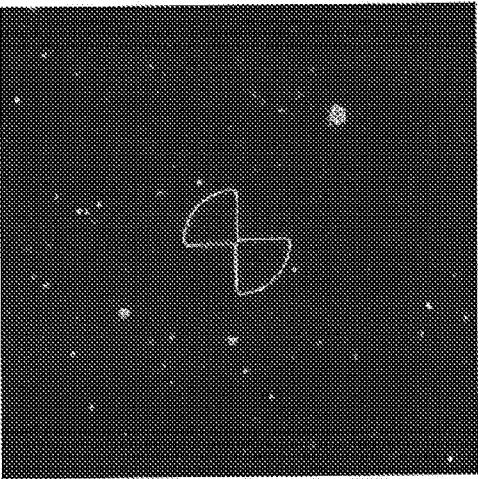
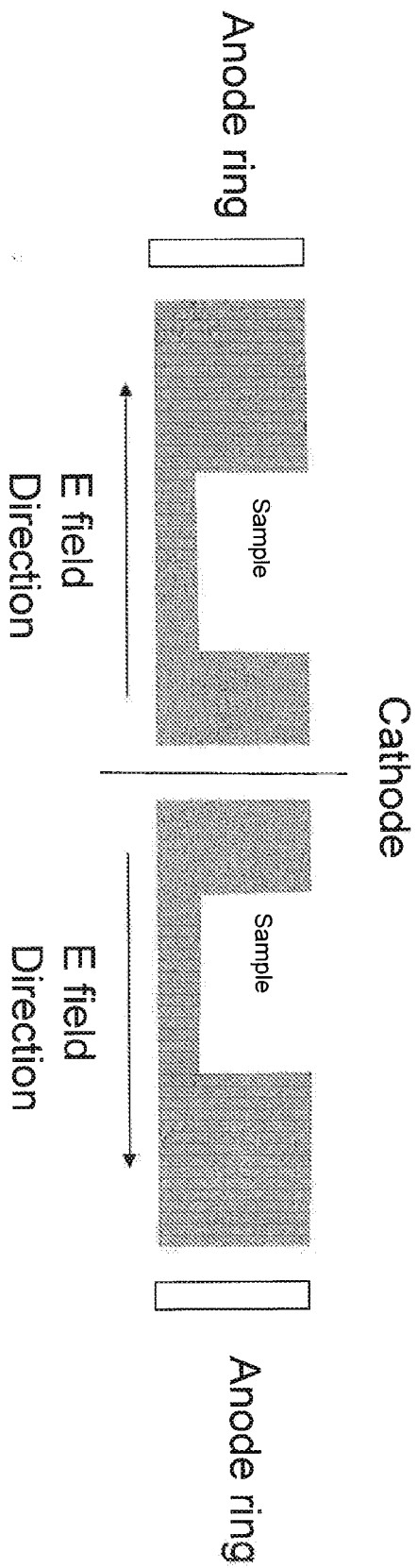


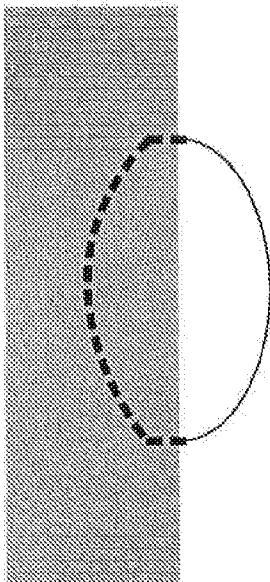
Fig. 7C

Cropped portion of microscopic image of
TREATED sample at $T = 360$
(everything stuck)

Fig 8. Circular Well Configuration (side view)

1. Media shown in grey
2. Ring of sample surrounds cathode (not shown w side view)
3. Run buffer sheath may flow over cathode to remove electrode by products
4. Configuration may be submerged or not submerged

Fig. 9: Tangential Flow Configuration (side view)



1. Media shown in grey
2. Hollow portion of media represented by dotted line
3. Tubing carrying recirculating sample shown in solid line.
May be pumped by peristaltic pump
4. Apply potential orthogonol to recirculating flow